FEBS Letters 546 (2003) 416–418

FEBS 27373

Correspondence

Expression of a family of expansin-like proteins during the development of *Dictyostelium discoideum*[1]

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First published online 2 June 2003

Dictyostelia have been extensively studied because of their fascinating life cycle, which involves both free-living single cells and multicellular aggregates that specialise into tissues under nutrient starvation. Dictyostelia could therefore be seen as a 'bridge' between the unicellular protists and multicellular organisms. Current molecular phylogenetic studies suggest that Dictyostelia sit somewhere at the foot of the division between the Animalia and the Fungi [1]. One obvious characteristic shared by plants and Dictyostelia is the presence of a cellulosic cell wall. Cellulose is made by a range of organisms including bacteria and even one group of metazoa (the tunicates). However, only a limited set of taxa use cellulose to produce a strong cell wall. These include the Stramenopiles (formerly classified as fungi) and algae as well as land plants and Dictyostelia [2]. In *Dictyostelium discoideum* the cell wall has particular significance in stalk cells during culmination when cells undergo rapid growth and vacuolation in a manner reminiscent of plant cell expansion. As well as their own cell walls, stalk cells also contribute cellulose to the communally synthesised stalk tube that encompasses them and determines their orientation of growth.

Plant cell walls are strong structures that also need to be extensible in order to allow expansion during cell growth. Expansins are proteins that mediate wall expansion in plants by disrupting non-covalent interactions between cellulose microfibrils and other polysaccharides that tether the microfibrils to one another [3]. As key mediators of cell expansion, expansins also appear to be important in plant morphogenesis [4]. Expansins are encoded by a multigene family with 38 members in *Arabidopsis thaliana* [5], but there is little or no evidence for expansins outside land plants. The exception to this is the presence of a small number of expansin-like sequences in the genome of *D. discoideum*.

Extensive searches of the Preliminary Directory of *Dictyostelium* Genes (http://genome.imb-jena.de/dictyostelium/) and tBLASTn and BLASTn searches of the *Dictyostelium* genome and EST databases revealed six expansin-like sequences (see supplementary data) that we name *Dictyostelium discoideum* expansin-like genes 1–6 (*Dd*ExpL1–6). Alignment of the *Dictyostelium* sequences with that of a typical plant expansin (*Cs*Exp1) is shown in Fig. 1A.

The *Dd*ExpL family generally show good similarity to plant expansins (for example, 19% sequence identity and 30% sequence similarity between *Dd*ExpL6 and *Cs*Exp1) incorporating most of the recognised elements that define these proteins. Expansins are secreted proteins and the N-terminus of a typical expansin contains a signal peptide with little conservation. Conservation among the sequences begins at the predicted mature protein around residue 35. The N-terminal halves of expansins are characterised by a series of conserved cystenyl residues, thought to be involved in maintaining the folded structure of the protein through disulfide bridge formation [5] and, apart from the pair at positions 94 and 103, all are present in the majority of the *Dictyostelium* sequences. The 'HFD box' is considered characteristic of expansins and is present in the *Dd*ExpL family, as are several of the conserved aromatic residues in the C-terminal half of the predicted proteins (residues 188–189, 217 and 219) some of which have been proposed to form a cellulose binding motif in the protein. Two of the *Dictyostelium* sequences diverge significantly from characteristic expansin sequences. *Dd*ExpL2 contains the sequence HLD rather than the characteristic HFD of expansins and has a much longer C-terminus than typical expansins. *Dd*ExpL4 appears to lack an N-terminal signal peptide and has the sequence HFG in place of the classical HFD motif. These features suggest that neither of these two putative proteins is likely to function as an expansin.

The developmental programme of *D. discoideum* from the onset of starvation to the production of spores takes roughly 26 h under normal laboratory conditions. During the first 10 h, the amoebae aggregate to form a mound containing on the order of 100 000 cells. These cells then take on a range of different roles to produce a 'slug' which can crawl across the substrate. By about 16 h after the onset of starvation, the slugs settle into a final position and the cells undergo differentiation with some forming a base, whilst others form a stalk that undergoes rapid elongation and is topped by a group of cells that form spores that are eventually released for dispersal.

Patterns of *Dd*ExpL transcript accumulation during the *Dictyostelium* life cycle are presented in Fig. 1B (see supplementary material for materials and methods). *Dd*ExpL2 was excluded from these studies as it was deemed unlikely to encode a protein with expansin activity. The top row of data are for IG7, a constitutively expressed gene used here as an RNA loading control [6]. Northern analyses for *Dd*ExpL1, 3 and 4 revealed that all appeared to be expressed at substantial levels throughout development with some slight variations. In contrast, *Dd*ExpL5 and 6 yielded no detectable signal suggesting their transcripts were absent or present at low abundance (data not shown). To increase detection sensitivity, reverse transcription polymerase chain reaction (RT-PCR) experiments were carried out on RNA from each stage. For *Dd*ExpL5 no RT-PCR product was detected suggesting this gene is not expressed at detectable levels under our conditions. We used PCR primers for *Dd*ExpL4 as controls for the RT-PCR experiments because no sequence information for IG7 is currently available and this gene showed fairly steady levels of transcript abundance throughout the time course in Northern

[1] Supplementary data associated with this article can be found at doi:10.1016/S0014-5793(03)00598-2

doi:10.1016/S0014-5793(03)00598-2

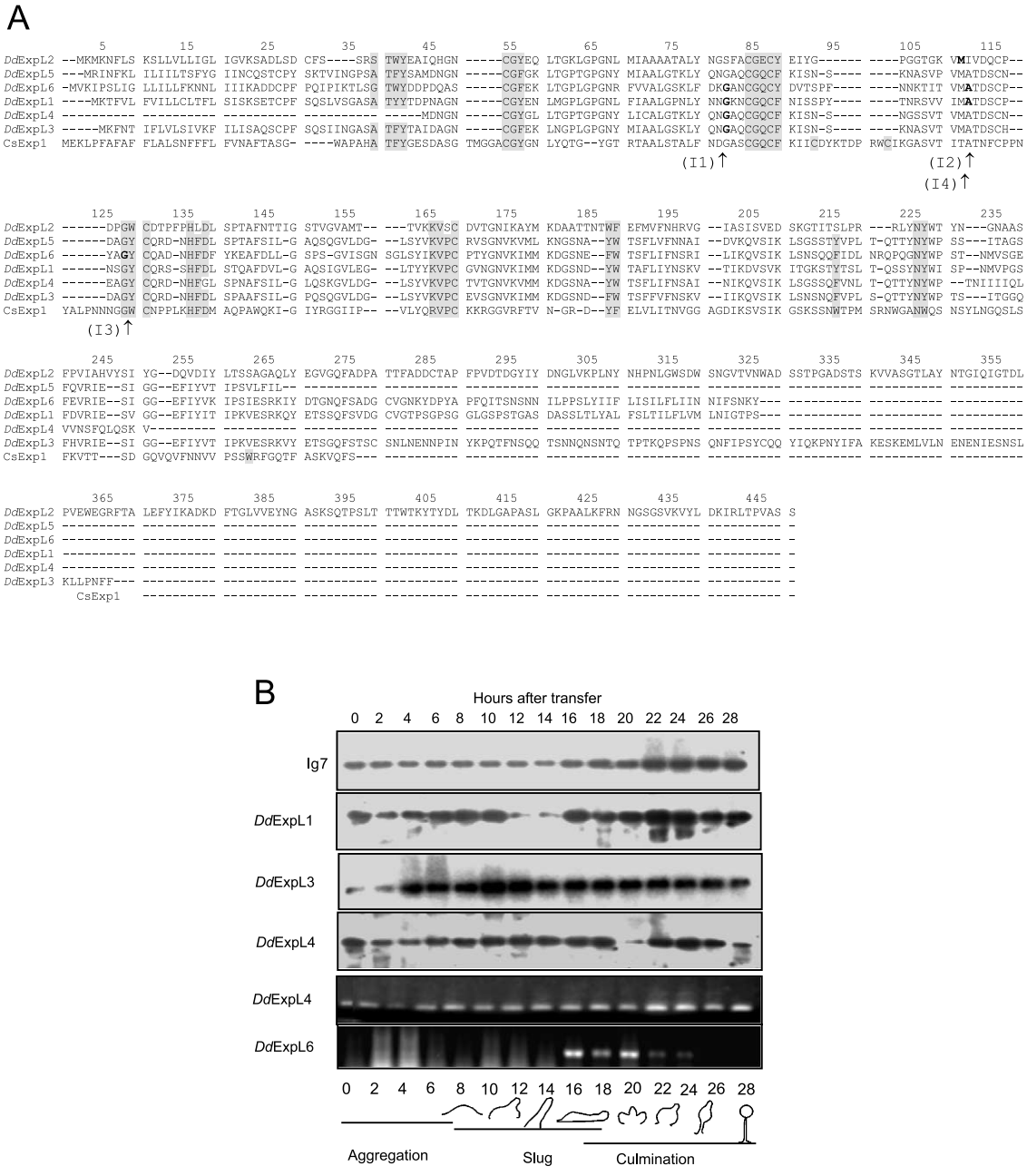

Fig. 1. A: Alignment of predicted protein sequences of the *Dd*ExpL gene family along with a representative plant expansin (*Cs*Exp1, from *Cucumis sativus*, accession number T10079). Retrieved sequences were aligned to the original alignment using 'profile alignment' in CLUSTALx. The shaded boxes indicate key regions of expansin protein conservation. Intron positions are indicated with an arrow and the point of intron insertion is indicated with bold typeface for those sequences in which the intron occurs. B: Detection of transcript abundance for *Dd*ExpL genes by Northern analysis and RT-PCR. RNA was isolated at 2-h intervals following transfer of cells to low nutrient 'starvation agarose'. 10 μg of total RNA was separated by electrophoresis and transferred to nitrocellulose filters before being hybridised with probes for different members of the *Dd*ExpL gene family. The top row is RNA probed for IG7, a constitutively expressed gene that serves as a control for RNA loading. RT-PCR products were separated by electrophoresis and visualised by staining with ethidium bromide and viewing under UV illumination.

analysis. Transcript for *Dd*ExpL6 was detected by RT-PCR and results from these experiments indicate that this gene is expressed between 16 and 24 h corresponding to late slug migration and the onset of culmination, when the stalk is first being formed.

These data are of interest when compared to the role of cellulose in *Dictyostelium* development. Once cells have aggregated to form a dome, they secrete mucopolysaccharides and cellulose and these are also the major constituents of the slime trail left by motile slugs [7]. Given the almost constitutive expression patterns of *Dd*ExpL1 and 3, we suggest the proteins encoded by these genes might play a role in maintaining the fluidity of cellulose containing secretions in this organism, although some expression is also apparent in free-living amoebae during aggregation, which are not reported to make cellulose. Cellulose is most prominent in the walls of elongating stalk cells and Blanton et al. [7] showed that cellulose synthase expression was highest at the onset of culmination when the

walls of stalk cells are first being produced. We suggest that *Dd*ExpL6 may play a specific role in the walls of these cells as its expression pattern closely mirrors that of the cellulose synthase gene. The elongation of stalk cells is highly reminiscent of that of plant cells where the walls have to be strong enough to provide mechanical support and yet extensible enough to allow expansion. In plant cell walls the extensibility of the strong wall appears to be facilitated by expansin action and we propose that *Dd*ExpL6 could have a similar function in *Dictyostelium*.

The presence of a family of expansin-like genes in *Dictyostelium* and plants suggests that these proteins must have been present in a common ancestor of these organisms but have only been retained in organisms possessing cellulose-based cell walls, as they do not (based on available sequence information) appear to be present in either the fungi or metazoa.

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